Cuticular hydrocarbons of the sunflower beetle, *Zygogramma exclamationis*

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Abstract

Hydrocarbons were the major lipid class on the cuticular surface of adults, nymphs, and eggs of the sunflower beetle, *Zygogramma exclamationis*, characterized by gas chromatography-mass spectrometry. Minor amounts of wax ester from 40 to 48 carbon atoms in size were only detected in larvae. The hydrocarbons ranged in size from 23 carbons (tricosene) to 56 carbons (trimethyltripentacontane) and were largely methylalkanes. The major components from females were 13,17,21-trimethylnonatriacontane (19%) and from larvae was *n*-nonacosane (17%). Males had 11,15- and 9,15-dimethylheptacosane (11%) and 13,17,21-trimethylnonatriacontane (11%) as the major components. In a sample of eggs, 13,17,21-nonatriacontane (16%) was the major component which was approximately 3 to 4-fold greater than the next most abundant hydrocarbons, dimethylheptacosanes, 2-methyloctacosane, methylnonacosanes, dimethyl- and tri-methylheptatriacontanes and dimethylnonatriacontanes.
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1. Introduction

The sunflower beetle, *Z. exclamationis* (Fitch) (Coleoptera: Chrysomelidae) occurs from Texas to Manitoba, Canada, and is the major defoliating pest of sunflower, *Helianthus annuus* L. (Westdal, 1975, Rogers, 1977, Charlet et al., 1997). The sunflower beetle has one generation per year, larvae and adults feed on cultivated sunflower and native *Helianthus* species. Adults feed during the day and consume the foliage beginning on the leaf margins, whereas larvae consume tissue over the entire surface of the leaf. Larvae are nocturnal feeders and congregate around the terminal portion of the plant during the day. The sunflower beetle overwinters as an adult, emerging from late May to early June. After mating, eggs are deposited on wild or volunteer sunflower until cultivated plants become available. Eggs are laid on the underside of leaves or on stems. Larvae develop through four instars and feed on plants from mid-June through late July. Larvae move off the plants and pupate in the soil, emerging as new-generation adults from late July to early August. These adults cause minimal damage to sunflower and exit the plants by mid-September to overwinter in the soil (Charlet, 1991, Charlet, 1992).

The adult sunflower beetle resembles the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), in size and

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color but only feeds on sunflowers. The Colorado potato beetle can have 1–3 generations a year, will feed on potato, tomato, eggplant, pepper and tobacco, or weeds such as nightshade and horse nettle, but not on sunflower. Both the species are Coleoptera, are native pests in the upper Midwest and because of their similar size and similar stripes on their elytra, they are often confused. Also, they are attacked by closely related parasitic flies. Recently, the cuticular surface hydrocarbons of the Colorado potato beetle have been characterized and found to contain abundant amounts of novel methyl-branched alkanes with a methyl branch on the second carbon of the carbon-chain backbone indicative of biosynthesis from methyl-branched amino acids (Nelson et al., 2003). We chose the sunflower beetle as a representative of a similar appearing insect to the Colorado potato beetle in order to determine the extent of similarity or diversity in the cuticular lipids.

Although management of sunflower beetle populations in commercial fields is generally through the use of insecticides, all stages are attacked by natural enemies, including both predators and parasitoids. The species causing the greatest mortality is the parasitic fly, *Myiopharus macellus* (Rheinhard) (Diptera: Tachinidae), which preferentially parasitizes the second instar of sunflower beetle larvae (Charlet, 1992). Attractants for parasitoids may be found in the cuticular lipids of their hosts and these attractants may be hydrocarbons (Nelson and Blomquist, 1995). In this report, we have characterized the hydrocarbon components found in the cuticular lipids of eggs, larvae and adult sunflower beetles.

2. Materials and methods

2.1. Insects

Adult males and females were collected by hand from sunflower plants in North Dakota in the summer of 1998. Second instar larvae were reared in the laboratory from eggs obtained from a colony of field collected adults.

2.2. Chemical analysis

Cuticular lipids were obtained by extraction of each sample (7–12 adults) by slurrying in 5 ml and then 2 ml of chloroform for 30 s each. The first two extracts were combined and the insects then were extracted a third time in the same manner to verify that all surface lipids had been removed. Larvae were extracted by placing them in a champagne funnel and rinsing with 6 ml chloroform over a 30 s interval. Eggs (100) were placed in a champagne funnel and rinsed with 5 ml chloroform for 30- s. Extracts were dried under nitrogen and resuspended in chloroform for analysis. The samples were analyzed for lipid classes on 10×10 cm thin-layer chromatography (TLC) plates of HPTLC-GHL (Analtech Inc., Newark, DE, USA) and developed in hexane:diethyl ether:formic acid (80:20:1, v:v:v). After drying, the developed plates were sprayed with 5% H_2SO_4 in 95% ethanol. After allowing the ethanol to evaporate, the lipid bands were visualized by heating the plate in an oven at 160 °C for 10 min to remove any residual solvents and then at 250 °C for 10–20 min to char the lipids. The hydrocarbon fraction was obtained from the total surface lipids by column chromatography on silica gel packed in a champagne funnel with hexane, pre-washed with 4 ml chloroform followed by 40 ml hexane. Samples were applied in 100–200 μl hexane and eluted with 8 ml each of hexane, 25% ether in hexane, 50% ether in hexane, 100% ether, and chloroform. The hydrocarbons were eluted in the hexane fraction.

A 1 μl aliquot of the samples in chloroform were analyzed both before and after chromatography on silica gel to verify if any major non-hydrocarbon components capable of being detected by GC–MS were being removed by the silica gel. All analyses were by gas chromatography-mass spectrometry (GC–MS) on a Hewlett Packard (HP) (Agilent Technologies, Wilmington, DE, USA) 5890A gas chromatograph equipped with a pressure programmable cool on-column injection port, an autoinjector, a 1 m retention gap connected to a 12.5 m $\times$ 0.2 mm capillary column of crosslinked dimethylsilicone Ultra 1 (HP), coupled to a HP 5970B quadrupole mass selective detector (Nelson et al., 2001). The carrier gas was He and the initial column temperature of 150 °C was programmed to 320 °C at 4 °C/min, and held at 320 °C until all components eluted. Hydrocarbon mass spectra were interpreted as previously described (Nelson, 1978; Blomquist et al., 1987; Nelson, 1993; Bernier et al., 1998; Carlson et al., 1998). The fatty acid moieties of the wax esters were determined by single ion monitoring (SIM) of the total ion current (TIC) data to extract the

Table 1
Micrograms ± standard deviation per insect of the components of the cuticular surface lipids as determined by GC–MS analysis ($n = 3$ for adults and 5 for larvae)

Lipid class	Males	Females	2nd Instar larvae
Hydrocarbons	64.4 ± 1.1	100.9 ± 10.1	1.0 ± 0.6
Wax esters	nd	nd	0.03 ± 0.03
Non-hydrocarbons	1.0 ± 0.1	1.5 ± 0.1	0.05 ± 0.07
Total	65	102	1

intensity of the ions corresponding to the protonated acid ion fragments from the wax esters (Nelson et al., 2000). For the saturated wax esters the protonated acid ions were m/z 257 for 16:0, m/z 285 for 18:0, etc., and for the unsaturated wax esters the acylium ions were at m/z 236 for 16:1, m/z 264 for 18:1, etc. The intensity of the ions was corrected for differences due to size of the fatty acid and/or wax ester and converted to a TIC value for the wax ester (Patel et al., 2001).

2.3. Lipid quantification

Total amount of cuticular lipid hydrocarbons, *n*-alkanes, methylalkanes and alkenes, were determined by GC–MS. Total ion current data were analyzed using a computer spreadsheet in Lotus123™ in which the detector response was corrected for lack of linearity by using a standard curve described by three equations (Nelson et al., 2001). The equations used for the ranges from 0 to 3 ng and from 100 to 1000 ng were linear, while the mid-range from 3 to 100 ng was best described by a first order polynomial equation. The formula in the spreadsheet selected the equation to be used based on the total ion current of the GC–MS peak being calculated, and used that equation to calculate the nanograms that each GC–MS peak represented.

3. Results and discussion

3.1. Gas chromatography–mass spectrometry

Thin-layer chromatography had shown that the majority of the cuticular surface lipid material of adults and second instar larvae was hydrocarbon with faint bands corresponding to triacylglycerols and material at the origin (these two faint bands were not characterized further). GC–MS analysis verified that the majority of the cuticular surface lipids were hydrocarbons (Table 1). Females had

almost twice as much surface hydrocarbons as did the males. Although females are generally larger this did not appear to fully explain the greater amount of surface hydrocarbons. First instar larvae had very little surface lipids. TLC analysis of the first instar larval surface lipid showed that hydrocarbons were also the major components (data not presented).

GC–MS analysis showed that males, females and second instar larvae had similar hydrocarbon components but in different proportions (Fig. 1 and Table 2). Hydrocarbons ranged in chain length from C23 to C54 (peak 51C) and had a bimodal distribution of the methyl-branched hydrocarbons consisting of a group from approximately C24 to C32 and a second group from approximately C36 to C46. In adults, *n*-alkanes, mono- and dimethyl-alkanes predominated in the first cluster of peaks in the bimodal distribution and internally branched di- and trimethylalkanes in the second cluster of peaks. Larvae had predominantly *n*-alkanes and 2-methylalkanes in the first cluster (Fig. 1c). Although the second cluster of peaks was less intense than the first cluster of peaks, both clusters were similar in composition to those of the adults.

Although infrequent, bimodal distributions of methyl-branched alkanes from the cuticular lipids of insects also have been observed in several other species: the Japanese beetle, *Popillia japonica* (Nelson et al., 1977), grasshopper, *Schistocerca vaga* (Nelson and Sukkestad, 1975), *Alphitobius diaperinus* (Lockey, 1979), the desert cicada, *Diceroprocta apache* (Hadley, 1980), the staphylinid beetle, *Trichopsenius frosti* (Howard et al., 1980), cowpea weevil, *Callosobruchus maculatus* (Baker and Nelson, 1981), the rove beetle, *Aleochara curtula* (Peschke and Metzler, 1987), phenotype II of the dampwood termite, *Zootermopsis* (Haverty et al., 1988), the tsetse, *Glossina tachinoides* (Nelson et al., 1988), North American cone beetles, *Conophthorus* (Page et al., 1990), the larval tobacco budworm, *Heliothis virescens* (Nelson and Buckner, 1995), the Formosan subterranean termite, *Coptotermes formosanus*, (Haverty et al., 1996), and *Reticulitermes* (Haverty and Nelson, 1997; Haverty et al., 1999). A bimodal distribution was seen in larval corn earworm, *Helicoverpa zea* (Nelson and Buckner, 1995), but not in cuticular hydrocarbons of adults (Carlson and Milstrey, 1991) or pupae (Buckner et al., 1996). Factors controlling the bimodal distribution of chain length in hydrocarbons are unknown.
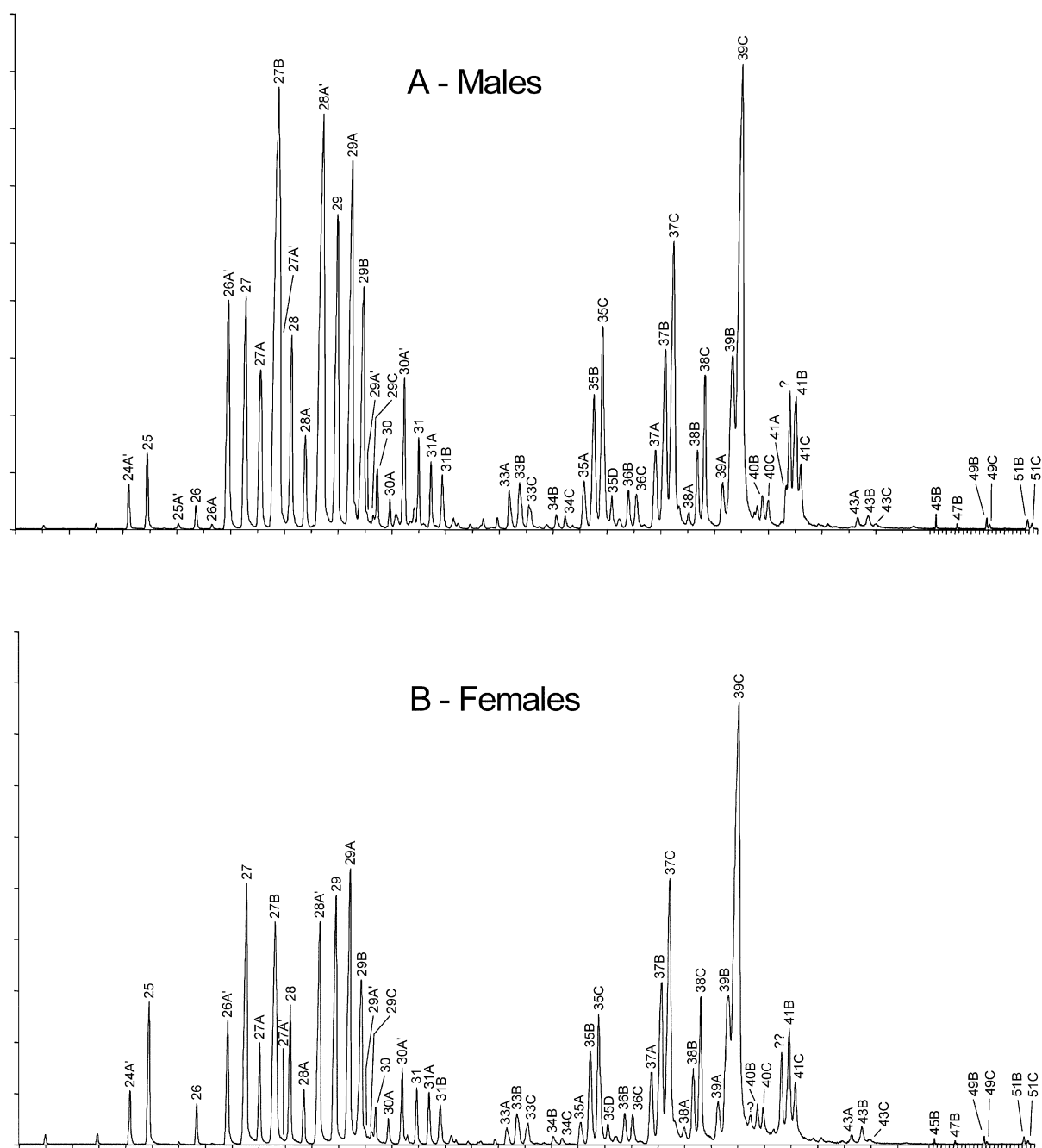
Fig. 1. GC–MS TIC traces of the total sample of the cuticular surface lipids of the sunflower beetle: (a) adult males; (b) adult females; (c) 2nd instar larvae and (d) the hydrocarbon fraction of the surface lipids of eggs. The numbers indicate the number of carbon atoms in the backbone of the molecule. The letters a, b, c and d indicate the presence of one, two, three and four methyl branches, respectively. A letter with a prime symbol means that the first methyl branch was on carbon 2 or 3 of the backbone of the molecule. WE = wax esters; NH = non-hydrocarbon. Both the WE and NH were removed from the hydrocarbons by column chromatography on silicic acid and elution with hexane.
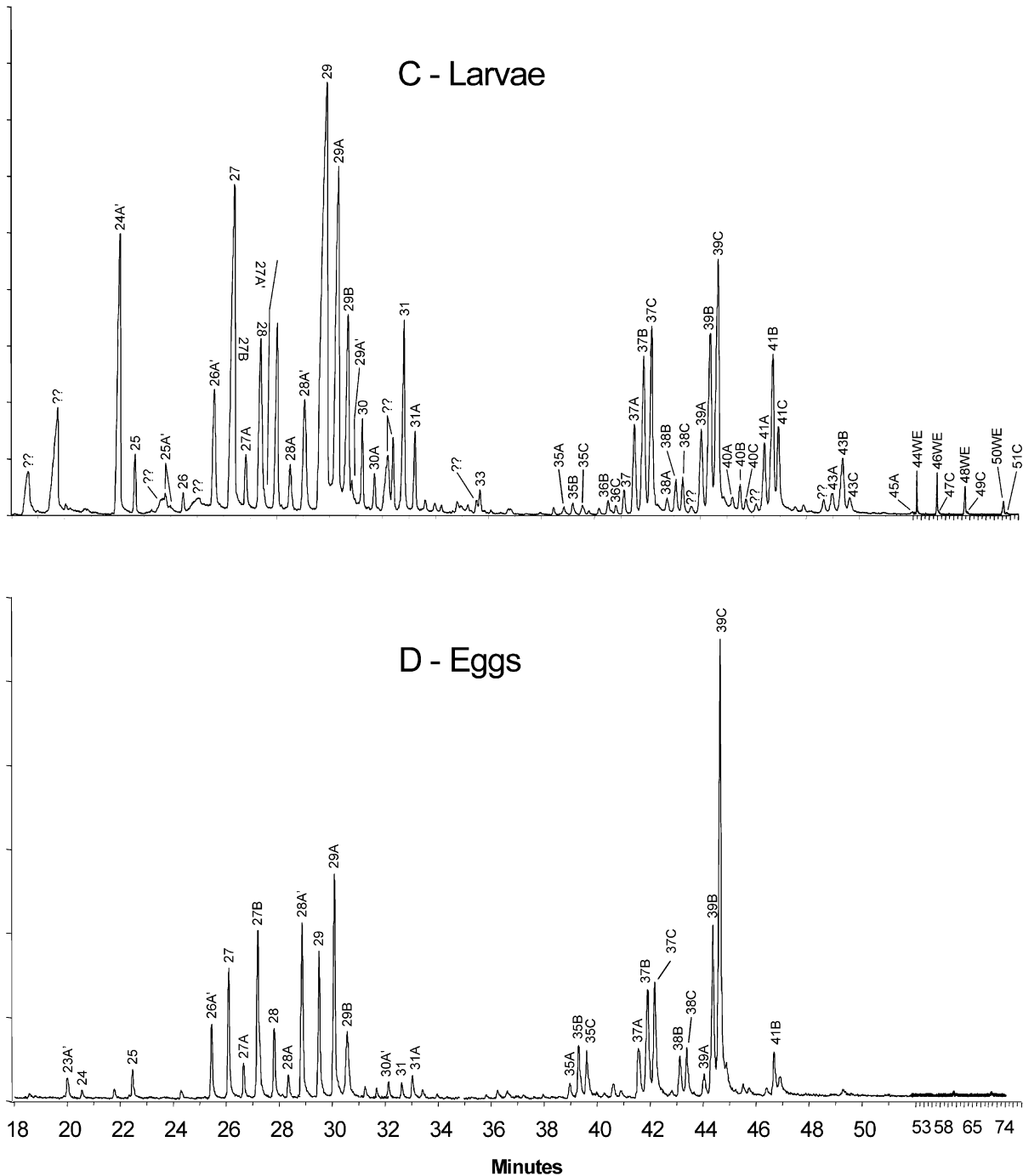
Fig. 1 (Continued).

However, 20-hydroxyecdysone is involved in the regulation of elongation of fatty acyl-CoA precursors for hydrocarbons in the housefly (Tillman et al., 1999).

The major hydrocarbons in males were GC–MS peaks 27B (11%), a mixture of 11,15- and 9,15-dimethylheptacosanes, and peak 39C (11%), 13,17,21-trimethylnonatriacontane (Table 2). The

Table 2
Percent composition from GC–MS analysis of the total cuticular surface lipids from males, females and larvae of the sunflower beetle[a]

GC–MS peak no.[b]	Carbon number	Kovats Indice	Males		Females		Larvae 2nd instar		
			Ave.	S.D.	Ave.	S.D.	Ave.	S.D.	Peak identification[c]
NH	–		–		–		0.4		NH
NH	–		–		–		2.0		NH
23	23	2300	0.1		0.2		–		n-tricosane
24	24	2400	0.1		0.2		–		n-tetracosane
24A′	25	2464	0.4		0.4		6.5	1.3	2-methyltetracosane
25	25	2500	0.7	0.1	1.5	0.2	0.4	0.2	n-pentacosane
25A	26	2534	t		–		t		13-methylpentacosane
25A′	26	2564	0.1		t		0.2	0.1	2-methylpentacosane
25A′	26	2571	–		–		0.1	0.1	3-methylpentacosane
26	26	2600	0.3		0.3		0.3		n-hexacosane
26A	27	2633	0.1		t		t		13-methylhexacosane
26A′	27	2664	3.1	0.2	1.2	0.1	2.2	0.3	2-methylhexacosane
27	27	2700	3.0		6.9	0.2	8.1	0.5	n-heptacosane
27A	28	2733	2.1	0.1	0.8	0.1	0.9	0.1	13-methylheptacosane
27B	29	2764	11.0	0.4	6.8	0.2	3.9	0.4	11,15- & 9,15-dimethylheptacosanes
27A′	28	2770	0.2		0.2		0.4	0.1	3-methylheptacosane
28	28	2800	1.9	0.1	1.2		3.1	0.5	n-octacosane
26 Ald	26	2815	0.1		0.1		–		hexacosanal
28A	29	2832	1.0		0.5		0.7	0.1	12- & 14-methyloctacosanes
28A′	29	2864	8.4	0.1	3.8	1.1	2.3	0.2	2-methyloctacosane
29	29	2900	4.2	0.2	6.1	0.2	17.5	0.8	n-nonacosane
29A	30	2933	5.8	0.1	6.9	0.2	8.8	0.2	15- & 13-methylnonacosanes
29B	31	2961	3.8	0.1	2.4	0.2	3.9	0.4	13,17- & 11,15-dimethylnonacosanes
29A′	30	2970	0.2		0.2		0.4	0.2	3-methylnonacosane
29C	32	2990	0.2		0.2		t		9,13,17- & 11,15,19-trimethylnonacosanes
30	30	3000	0.5		0.3		1.1	0.3	n-triacontane
28 Ald	28	3014	t		–		–		octacosanal
30A	31	3031	0.3		0.2	0.1	0.5	0.1	14-methyltriacontane
NH,30B,A′	32,31	3064	1.3		0.5	0.2	0.4	0.2	NH[L1] & dimethyl-[T] & 2-methyltriacontane
NH	–	3076	0.3		0.3	0.3	0.5	0.1	NH[2]
31	31	3100	0.7		0.4		2.5	0.4	n-hentriacontane
31A	32	3130	0.6		0.4		0.9	0.1	15[*]-, 13-, 11- & 9-methylhentriacontanes
31B	33	3159	0.6		0.4		0.2		13,17- & 11,15-dimethylhentriacontanes
31C	34	3187	0.2		0.1		t		9,13,17- & 11,15,??-trimethylhentriacontanes
32	32	3200	0.1		0.1		t	0.1	n-dotriacontane
32A	33	3231	0.1		0.1		t		16-methyldotriacontane
32B	34	3257	0.1		t		–		12,16-dimethyldotriacontane
32A′	33	3264	0.2		0.1		–		2-methyldotriacontane
32C	?	3284	t		t		–		?
33	33	3300	0.2		0.1		0.2	0.1	n-tritriacontane

Table 2 (*Continued*)

GC–MS peak no.[b]	Carbon number	Kovats Indice	Males		Females		Larvae 2nd instar		
			Ave.	S.D.	Ave.	S.D.	Ave.	S.D.	Peak identification[c]
33A	34	3329	0.4		0.2		0.1		17-, 13-, 11- & 9-methyltritriacontanes
33B	35	3354	0.6		0.4	0.1	t		13,17-, 13,19-, 11,15- & 9,13-dimethyltritriacontanes[L?]
33C	36	3379	0.4		0.3		t		13,17,21-, 11,15,19- & 9,13,17-trimethyltritriacontanes[L?]
33D	37		0.1		t		t		?
34A	35		0.1		t		–		12- & ??-methyltetratriacontanes
34B	36		0.3		0.2		t		13,17- & 12,16-*dimethyltetratriacontanes[L?]
34C	37		0.2		0.1		t		13,17,21- & ??-trimethyltetratriacontanes[L?]
35	35	3500	0.1		t		0.1		*n*-pentatriacontane[M?,F]
35A	36	3526	0.6		0.3		0.1	0.1	17-*, 15-, 13-, 11-,9-methylpentatriacontanes
35B	37		1.8		1.1	0.1	0.2		15,19-[L*], 13,17-* & 11,15-dimethylpentatriacontanes
35C	38	3582	2.8	0.2	1.7	0.1	0.2	0.1	13,17,21-trimethylpentatriacontane[L?]
35D	39		0.4		0.2		0.1		9,13,17,21-tetramethylpentatriacontane[L?]
36A	37		0.2		0.2		0.1		?
36B	38		0.5		0.4		0.3	0.1	12,16-* & ??-dimethylhexatriacontane
36C	39		0.5		0.4		0.2		14,?,?-, 13,?,?-, 12,16,20- & 11,?,?-trimethylhexatriacontanes
36D	40		0.1		0.1		0.3	0.2	?
37A	38		1.2	0.1	1.0	0.1	1.0	0.4	17-[M*,F*,L*], 15-, 13- & 11-[M*,F*]methylheptatriacontanes
37B	39	3758	2.9	0.1	3.7	1.0	2.9	0.1	13,21-, 13,17-* & 11,17-dimethylheptatriacontanes
37C	40	3782	4.8	0.1	9.3	1.1	3.5	0.6	13,17,21-[F*,L*] & 11,15,??[Lt]-trimethylheptatriacontanes
37D	41		0.4		0.3	0.1	0.3	0.2	?
38A	39		0.3		0.3		0.3	0.1	18- to 12-[M,F]methyloctatriacontanes
38B	40		0.9		0.9		0.6	0.1	13,17-, 12,18- & 12,16-dimethyloctatriacontanes
38C	41		1.8		1.8		0.6	0.1	13,17,21- & 12,16,20-[M*,F*]trimethyloctatriacontanes
38D	42		–		–		0.2		?
39A	40		0.8		0.6	0.1	1.3	0.1	19-, 17-, 15-, 13-*, 11- & 9-methylnonatriacontanes
39B	41		3.7	0.1	6.4	0.3	3.8	0.3	13,17-, 13,19- & 13,21-[M*,F*]dimethylnonatriacontanes
39C	42	3977	11.0	0.4	19.4	0.8	6.5	0.7	13,17,21-trimethylnonatriacontane
39D	43		0.1	0.1	–		0.7	0.2	?
40A	41		0.2	0.1	–		0.4	0.1	12-methyltetracontane
?			0.3		0.4	0.1	–		NH[3]
40B	42		0.4		0.4	0.1	0.5	0.1	13,?? & 12,??-dimethyltetracontanes
40C	43		0.4		0.4	0.1	0.4	0.1	14,18,22-* & ??-trimethyltetracontane
NH			t		t		0.1		NH[4]
NH,41A	42		0.5	0.1	t		1.1	0.1	NH[4] & 13-methylhentetracontane
NH,41B	43		2.5	0.1	2.7	0.2	2.6	0.2	NH[5] & 13,19-, 13,21-[F*L*] & 13,23-dimethylhentetracontanes
41C	44		0.9	0.1	1.0		2.0	0.3	13,17,21-*, 13,19,23-, 13,21,25- & 11,??,??-trimethylhentetracontanes
42A	43		0.1		0.1		0.2		14-, 13-* & 12-methyldotetracontanes
42B	44		0.2		0.1		0.2	0.1	14,??-, 13,21-, 12,20- & 11,??-dimethyldotetracontanes
42C	45		t		0.1		t		?
NH			t		0.1		0.2		NH[4]
43A	44		0.3		0.2		0.4	0.1	13-methyltritetracontane
43B	45		0.3		0.3		0.8	0.1	13,21-*, 13,23-, 13,25- & 11,??-dimethyltritetracontanes

Table 2 (*Continued*)

GC–MS peak no.[b]	Carbon number	Kovats Indice	Males		Females		Larvae 2nd instar		
			Ave.	S.D.	Ave.	S.D.	Ave.	S.D.	Peak identification[c]
43C	46		0.2		0.2		0.5	0.1	13,17,21-L*, 13,21,25-, 13,??,??- & 11,??,??-trimethyltritetracontanes
44A	45		t		t		–		?
44B	46		0.1		t		0.1	0.1	12,??-dimethyltetratetracontane
45A	46		0.1		t		t		13-methylpentatetracontane
45B	47		0.3		0.2		0.1	0.1	13,17-L*, 13,19-M*,F*dimethylpentatetracontanes
45C	48		–		–		0.1	0.1	?
46B	48		0.1		t		t		13,??-dimethylhexatriacontane
47A	48		t		t		–		13-methylheptatetracontane
47B	49		0.2		0.1	0.1	0.1	0.1	13,21-dimethylheptatetracontane
47C	50		0.1		t		0.1		13,17,21-trimethylheptatriacontane
48A	49		t		t		–		?
48B	50		t		0.1	0.1	t		?
49B	51		0.4		0.2		0.2		13,21- & 13,23-dimethylnonatetracontanes
49C	52		0.3		0.1	0.1	0.2		13,19,23- & ??,??-trimethylnonatetracontane
50B	52		0.1		t		–		12,??- & 13,??-dimethylpentacontanes
50C	53		0.1		0.1	0.1	–		?
51B	53	5144	0.5		0.3		0.4	0.1	13,23- & 13,25-dimethylhenpentacontanes
51C	54	5164	0.3		0.2		0.2		13,23,27- & 13,21,25-trimethylhenpentacontanes
?			0.1		t		–		?
52B	54		–		–		–		?
52C	55		–		–		–		?
53B	55		0.1	0.1	t		–		?
53C	56		0.1	0.1	t		–		?

[a] The wax esters of the larvae were not included. Values are averages and standard deviations. A 't' means the peak was present at less than 0.05%. Where no standard deviation is listed, the value was less than 0.05% ($n=3$ for adults and 5 for larvae).

[b] The GC–MS peaks correspond to those marked in Fig. 1. The number is the number of carbon atoms in the backbone of the molecule. The letters A, B, C and D indicate one, two, three and four methyl branches, respectively. A letter with a prime symbol means that one of the methyl branches is near the end of the molecule, i.e. on carbon 2, 3 or 4. Therefore, it is possible to have two or three peaks in sequence marked with a prime symbol, e.g. an eluting sequence of 4-, 2- and 3-methylalkanes. Where a peak is multi-component, the compounds are listed in their order of elution as determined by examining individual scans throughout the peak. If the major component could be estimated, it is marked with an asterisk. NH=non-hydrocarbon; non-polar but slightly more polar than the hydrocarbons and could be removed by chromatography on silicic acid while the hydrocarbons were eluted with hexane.

[c] Structures for the individual hydrocarbons were based on the combination of their mass spectra, retention time and feasibility of biosynthesis. If the major isomer could be determined, it is marked with an asterisk. If the major isomer was different between samples, it is marked with an M* (=males), F* (=females), or L* (=larvae). If an isomer was present only in one or two samples, that isomer is marked with an M (=males), F (=females), or L (=larvae). A '?' indicates the identity could not be determined from the mass spectra and the best indication of the compound is based on the GC-MS Peak No. Non-Hydrocarbon footnotes: L1 – only in larvae, base peak at m/z 239, and had an 87% match with 5,7-dihydroxy-3,6,8-trimethoxyflavone from the Wiley275 mass spectra data base; 2 – base peak at m/z 239 and had a 50% match with 2-chloro-4,6-di(t-pentyl)-phenol but with no Cl isotope ion; 3 – was not in larvae and was characterized by ions at m/z 171, 197 and 211 in a mass spectrum similar to that of a methylalkane; 4 – had a major or base peak at m/z 267; 5 – was characterized by ions at m/z 169, 185 and 211.

Table 3
Percent distribution of the hydrocarbon classes in the cuticular surface lipids of males, females and larvae ($n = 3$ for adults and 5 for larvae)

Hydrocarbon class	Males	Females	2nd instar larvae
n-alkanes	13	18	33
Monomethylalkanes	15	12	16
Dimethylalkanes	30	26	21
Trimethylalkanes	25	35	14
Tetramethylalkanes	1	1	1
2-Methylalkanes	14	6	11
3-Methylalkanes	<1	<1	1
Unknowns	1	1	3

major hydrocarbon in females also was peak 39C (19%), but in larvae was peak 29 (17%), *n*-nonacosane. The major methyl branched hydrocarbon in larvae was peak 29A (8.8%), 13- and 11-methylnonacosanes. The major lipid classes were the internally branched dimethylalkanes and trimethylalkanes in adult males and females, but were the *n*-alkanes and dimethylalkanes in larvae

(Table 3). The proportion of 2-methylalkanes in females was only half that found in the hydrocarbons of the males and larvae.

A sample of 100 eggs was analyzed by GC–MS after passage through a column of silica gel. The dominant hydrocarbon was peak 39C, 13,17,21-trimethylnonatriacontane (16%) (Fig. 1d). This component was approximately 3 to 4-fold greater than the next most abundant egg hydrocarbons, dimethylheptacosanes (5%), 2-methyloctacosane (4%), methylnonacosanes (5%), dimethylheptatriacontanes (4%), trimethylheptatriacontanes (4%) and dimethylnonatriacontanes (5%). Neither wax esters nor large amounts of *n*-alkanes were found in the egg sample.

Even carbon-numbered wax esters from C44 to C50 were found in larvae (Fig. 1c) but no wax esters were detected in the adult cuticular surface lipids. The percent composition of the larval wax esters was: C40, 2.5 ± 2.1; C42, 8.6 ± 3.4; C44, 24.3 ± 3.0; C46, 28.0 ± 1.7; C48, 25.5 ± 2.8; C50,
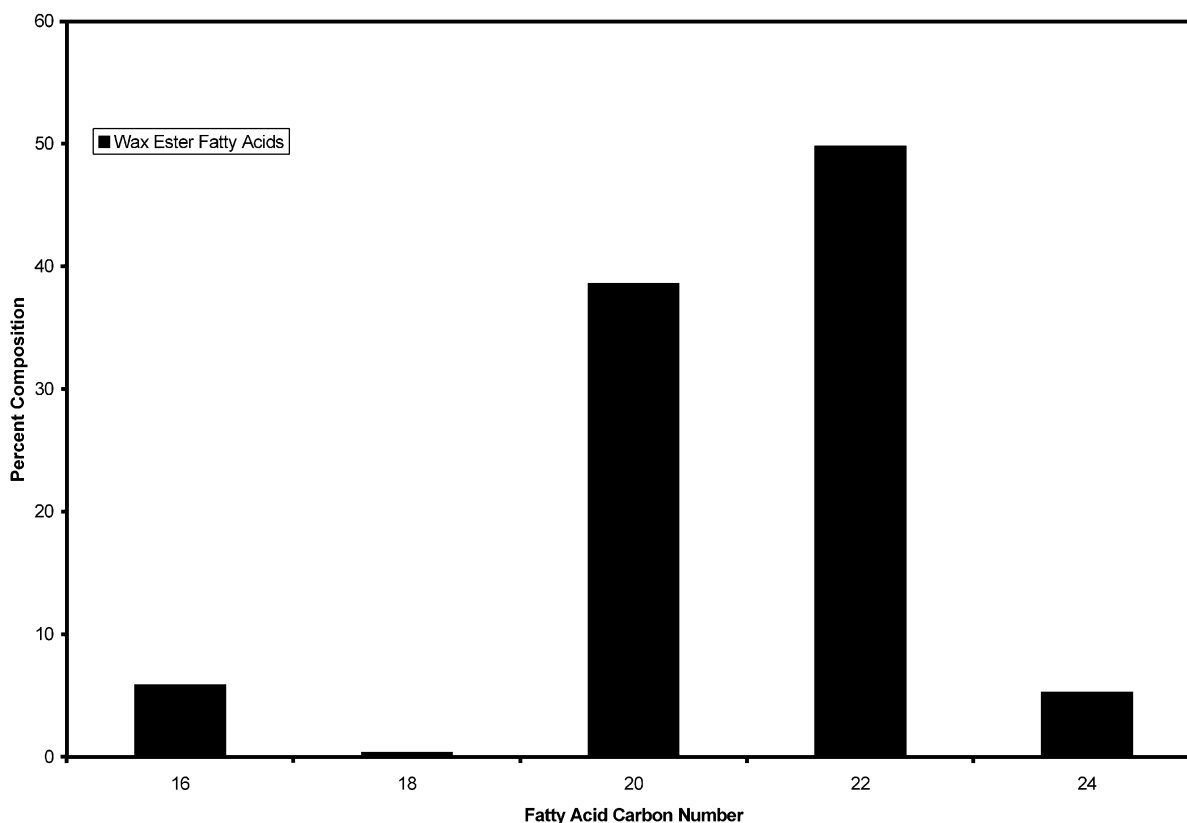

Fig. 2. Fatty acid composition of the total wax esters from larval surface lipids determined from SIM of the TIC fragment ions corresponding to the protonated acid moieties.
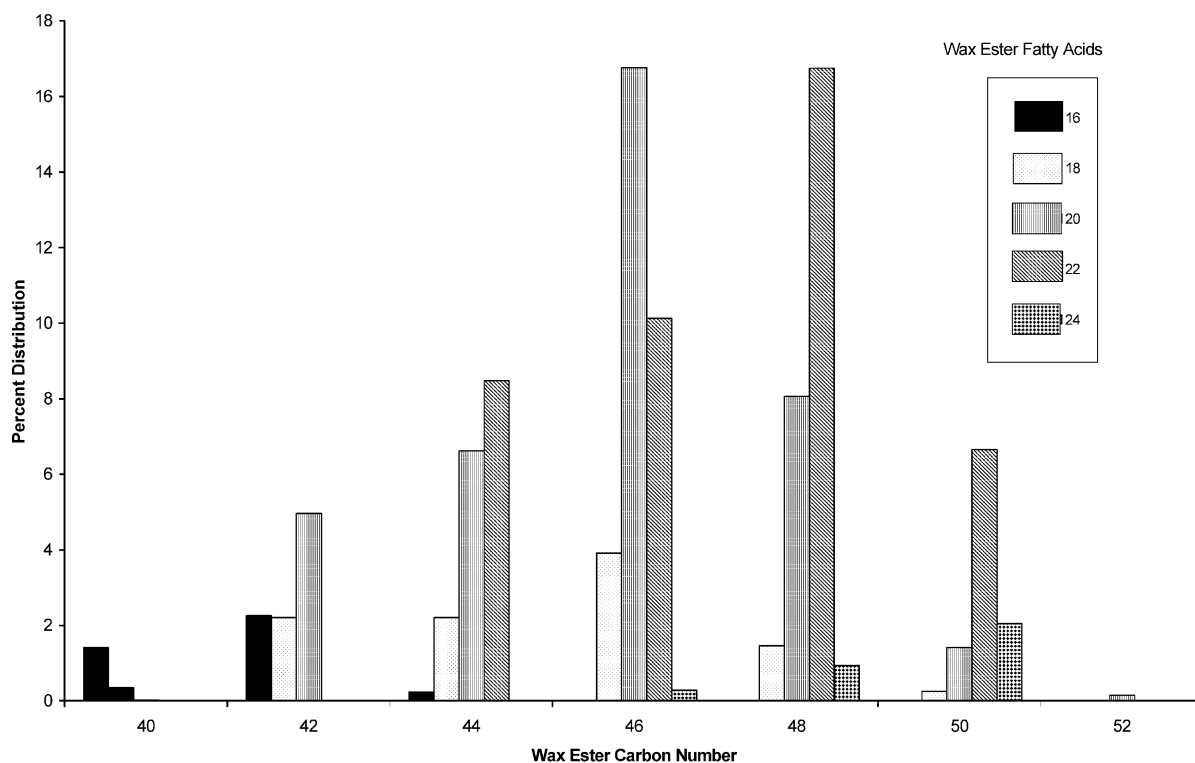
Fig. 3. Distribution of the fatty acid moieties within the individual wax esters from the larval surface lipids.

10.1 ± 7.2; and C52, 0.4 ± 0.7. The fatty acid moieties were even-numbered and ranged from C16 to C24: C16, 5.9 ± 3.6; C18, 0.4 ± 0.7; C20, 38.6 ± 4.8; C22, 49.8 ± 6.6; and C24, 5.3 ± 4.6 percent (Fig. 2). Each wax ester peak was a mixture of isomers and the alcohol moieties were straight-chain. The distribution of the fatty acid moieties within each wax ester peak is shown in Fig. 3.

Contamination of the larval surface lipids by plant waxes may occur. The source of the wax esters is unknown but may be from the plant material eaten, or contamination from the plant on the cuticle of the larvae. An analysis of a hexane rinse of sunflower leaves showed the same profile of wax esters as found in the larval samples. The sunflower wax esters also had the same distribution of fatty acids within each wax ester peak as found in the wax esters from the surface of the larvae. Also, larvae had a higher proportion of *n*-alkanes than adults did and the major *n*-alkanes were heptacosane and nonacosane (Table 2). These two *n*-alkanes were also the major *n*-alkanes in the waxes from the sunflower leaf. There was no

obvious indication of contamination of adult surface lipids by plant waxes.

A beetle in the same geographic area and which resembles the sunflower beetle is the Colorado potato beetle, *L. decemlineata*. The public sometimes confuses them, but they have different and non-overlapping host ranges. The major components of the cuticular surface lipids of the Colorado potato beetle are saturated hydrocarbons (Szafranek et al., 1994). These beetles do not have a bimodal distribution of their hydrocarbons, have undetectable to trace amounts of *n*-alkanes, and over 50% of their hydrocarbons are mono-, di- and trimethyl-branched alkanes with a methyl branch on carbon atom 2 of the backbone of the molecule (Nelson et al., 2003). Such structures require the primer for their biosynthesis that can be derived from the amino acids, valine and leucine. There is no need for amino acids to be involved in the biosynthesis of the methylalkanes of the sunflower beetle because they have no methylalkanes with an even number of carbons between the branch points. An elucidation of the differences in the nutritional requirements and

biosynthetic pathways of these two beetles may be very enlightening. Kairomones are used by many parasitoids in host location and these include host salivary gland or mandibular secretions, host frass, honeydew and cuticular secretions (Jervis and Kidd, 1996). Non-volatile cuticular lipids may be involved in host recognition by the parasitoid as well as in discrimination of apparent similar hosts so that the most preferred host is selected. Also, in artificial rearing including appropriate hydrocarbons in or on the diet may aid in preconditioning the emerging parasitoid to better recognize a suitable host. Additional studies are needed to determine if the primary parasitoid of the sunflower beetle, *M. macellus*, utilizes the hydrocarbon components of the surface lipids of the second instar larvae as chemical cues in host finding.

Acknowledgments

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